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BIODEGRADATION OF CHLORINATED ORGANIC COMPOUNDS

Robert S. Ingols, et al

Georgia Institute of Technology

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FINAL REPORT

PROJECT NO. B-228

BIODEGRADATION OF CHLORINATED ORGANIC COMPOUNDS

By Robert S. Ingols and Paula C. Stevenson

Prepared for
National Institutes of Health
Bethesda, Maryland

Grant-In-Aid WP-188-04 March 1963

Engineering Experiment Station
GEORGIA INSTITUTE OF TECHNOLOGY
Atlanta, Georgia

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Atlanta, Georgia

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Robert S. Ingols
Paula C. Stevenson

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BIODEGRADATION OF CHLORINATED ORGANIC COMPOUNDS

by

R. S. Ingols and P. C. Stevenson*

The use of economic poisons (a biocide applied for the economic benefit of the user) is increasing rapidly. Many of these economic poisons are organic compounds which have a large number of carbon-chlorine bonds, such as pentachlorophenol, which is used for the control of mildew on textiles and of slimes or molds in heat exchange systems, and chlorodane, a heavily chlorinated, polycyclic organic compound.

While economic poisons frequently serve well the purposes for which they are used, they sometimes have undesirable side effects. Wallace (10) has observed that a large number of birds are killed by insecticides used for the control of those insects which are the normal food of the birds. The birds may consume either dead or living, but partially affected insects, both of which contribute to the poison level in the bird. A large number of fish kills which have been reported by the State Water Pollution Control authorities to the Public Health Service (8) have been attributed to agricultural use of economic poisons in the control of insects.

Fortunately, the economic poisons are subject to some biological degradation. Milo (4) has reported that the waste from a chemical industry producing economic poisons can be treated on trickling filters. The question arises, what happens to the organic compounds that are destroyed? An answer is given by

*Research Professor and Research Assistant, Engineering Experiment Station, Georgia Institute of Technology, Atlanta, Georgia

Powers (7) that an increase in chloride ion concentration develops as a result of the biological activity.

In part, this study is an outgrowth of the senior author's earlier interest in the effect of a dose of chlorine on the BOD of a sewage plant effluent. In an article published by Ingols and Jacobs (2), it was indicated that chlorine (hypochlorous acid) which has been added to the organic components of the sample to be analyzed frequently reduces the BOD of the sample to a far greater extent than could be expected from the purely oxidative effect of the chlorine atoms which have reacted with the organic food. The research by Ingols and Jacobs was an attempt to find why 1 mg/l chlorine reduced the BOD of sewage by 2 mg/l when 1 mg/l chlorine is chemically equivalent to only 0.23 mg/l oxygen. This eightfold increase in the BOD reduction over its oxidative capacity indicated that there is some biological factor that is not the result of purely oxidative change similar to common biological reactions; it was thought that there might be biological inhibition from an addition or substitution of chlorine onto the organic molecule. This was indicated by the change in the biological availability of certain chlorinated organic residues.

Tyrosine was chosen as one of the compounds to be studied; this amino acid has a phenol ring and takes on chlorine readily. When one millimole of tyrosine is reacted with one millimole of chlorine, the BOD of the product is quickly reduced to zero; this reduction is far in excess of the extent of the oxidation of the tyrosine molecule by the chlorine as known from the molar ratio of chlorine added to the tyrosine. This fact indicated that when the chlorine

atom has reacted with the tyrosine molecule, the organic residue fraction becomes virtually unavailable to the normal flora or innoculum (seed) of the BOD test. When this study was extended to include phenol, similar low BOD results were obtained. When 5 mg/l phenol is reacted with 5 mg/l chlorine, a mixture of either 2,4-dichlorophenol or trichlorophenol is produced. The BOD of the chlorinated phenol approaches zero even though there is only 9 per cent oxidative change of the phenol in the formation of trichlorophenol. At the time the BOD study was published, the senior author was not familiar with the article by Milo (4) and concluded incorrectly that the carbon-chlorine bond could not be degraded. Subsequent work by Powers (7) and by the authors indicates that these compounds are available to bacteria and that the carbon-chlorine bond can be broken under certain conditions. It is the purpose of this paper to indicate some of these conditions.

Powers (7) indicates that the chlorophenols which are added to the waste water from the manufacturing plant for sodium pentachlorophenate may be destroyed by an aeration procedure in the waste treatment plant. It has been observed by the senior author that when the sodium pentachlorophenate is used for the control of slime in certain industrial applications, eventually the slime organisms develop and another slime controlling compound must be used. These observations indicate that eventually organisms can develop or build an immunity and destroy sodium pentachlorophenate as an effective economic poison.

PRESENT STUDY

An "activated" sludge was originally sent to us by Dow Chemical Company but subsequently was developed directly by aerating the soil with glucose

and peptone in a mineral nutrient medium free of added chloride ions. This sludge was very light yellow-tan in color, and did not break down the chlorinated phenolic compounds in the presence of glucose and peptone. Acclimation of the sludge was brought about by withdrawing the normal food, peptone and glucose, while maintaining a constant level of chlorinated phenol over a period of several months. During the withdrawal of food, the glucose-peptone sludge undergoes a decided change in appearance, becoming dark brown in color. After the sludge has been used to degrade a chlorinated phenolic compound it is refed with peptone and glucose until its next use.

During our investigations we used the following methods to determine the degradation of the phenol compound:

1. A modification of the 4-aminoantipyrine methods for phenols which was described by Ochynski, F. W., The Analyst 85, 278 (April 1960).
2. Ultraviolet absorption spectra of the compounds recorded on the Beckman model DK-2 spectrophotometer.
3. The mercuric nitrate method for chloride ion concentration from Standard Methods for the Examination of Water and Waste Water 11th Edition, American Public Health Association, New York City (1960).

After a correlation between the spectrophotometric recordings and the 4-aminoantipyrine method for phenol was established, the latter method was discontinued due to the greater time required for this analysis. At present we are using

the ultraviolet adsorption spectra to determine phenol change and/or disappearance and the mercuric nitrate method to determine the amount of chloride ion produced.

Nine different chlorinated phenolic compounds and phenol derivatives have been investigated using the above methods. They are: (1) ortho-chlorophenol, (2) meta-chlorophenol, (3) para-chlorophenol, (4) 2,4-dichlorophenol, (5) 2,5-dichlorophenol, (6) 2,4,6-trichlorophenol, (7) sodium pentachlorophenate, (8) dichlorohydroquinone, and (9) 2,5-dichloro-para-benzoquinone. Our general procedure for investigation is as follows: Samples of one liter are made up containing the compound to be investigated, mineral nutrients, and a large amount of acclimated sludge maintained with glucose and peptone. Aliquots of the supernatant are withdrawn for analysis; these are passed through a millipore filter in order to remove the organisms, and the filtrate is refrigerated until the end of the experimental period. The chloride ion tests are generally made on a day-to-day basis. The ultraviolet absorption spectra are recorded on a single chart at the end of the experimental period so that a direct observation of the extent of degradation of the phenolic compounds over a 3 to 5 day period is obtained.

Only one aspect of our general procedure has been modified since the investigations began. Originally a 5 liter wide-mouthed bottle was used as the aeration vessel, but recently one liter graduated cylinders have been used.

After establishing a general procedure, investigations were begun to determine the optimum conditions for degradation. The effect of variations in pH, temperature, and the phenolic compound concentration was determined. Ranges

used were pH 6.1 to 9.3, temperature 10° to 40°C and concentrations of 50 to 400 mg/liter. The results of the pH range tests at several temperatures, as shown in Table I, indicate that a pH of 6.8 to 7.0 and temperature of 26° C were optimum; the concentration of 100 mg/l of several phenolic compounds was found, as shown in Table II, to be the optimum concentration.

Our results are still somewhat inconclusive. Of all the compounds tested to date, we have successfully degraded the ortho-, meta- and para-monochlorophenols and the 2,4-dichlorophenol. The remaining compounds, except for sodium pentachlorophenate, have been degraded partially, after several days, with as high as 75 per cent chloride ion production for the 2,4,6-trichlorophenol. Of all the compounds tested, the sodium pentachlorophenate is the only one which has resisted degradation at the concentrations studied. As mentioned earlier, the sludge had to be acclimated over a period of several months. Once this had taken place, however, the degree of reactivation (with the compound to be studied) necessary for total degradation seemed to depend on the compound itself. At first it was found that the monochlorophenols did not require that the sludge be reactivated prior to each trial whereas the 2,4-dichlorophenol and trichlorophenol indicated a definite requirement for the sludge to be reactivated (Table III). However, subsequent work indicated that it was not necessary to reactivate the sludge with anything other than the glucose, peptone, and mineral nutrients (Table IV). Another factor, also mentioned earlier, which changed our results was the use of narrow vessels for better sludge distribution. Prior to their use, only 100 per cent disappearance of phenol was obtained without

TABLE I

OPTIMUM pH AND TEMPERATURE

1. Ortho-Chlorophenol

% Disappearance of Phenol Ring

Temp. °C	Days (Aerated)	Conc. mg/l	pH Range 6.5	7.3	7.5	7.9
20	1	200	33	27	19	20
	2	200	67	46	37	50
	3	200	55	54	37	40
27	1	200	88	89	48	91
	2	200	100	100	90	100
	3	200	100	100	100	100

			pH Range 6.8	8.4	9.0	9.3
25	1	200	74	22	6	3
	2	200	90	43	27	18
	3	200	97	95	76	64
30	1	200	57	6	0	0
	2	200	76	24	9	0
	3	200	94	45	15	0

2. Meta-Chlorophenol - 26°C

			pH Range 6.1	7.0	8.0	8.5
% Disappearance	2	200	5	0	0	0
	3	200	20	40	0	0

3. 2,4,6-Trichlorophenol - 26°C

			pH Range 6.5	7.0	8.5	8.8
% Disappearance	1	200	25	100	100	100
	2	200	100	-	-	-
% Chloride Ion	1	200	2	15	7	7
	2	200	5	15	7	13
	3-1/2	200	9	27	13	13

TABLE II

OPTIMUM CONCENTRATION

Compound	Days (Aerated)	Concentration (mg/l) 50	100	200	400
		Neutral pH (7) and Room Temperature (26°C)			
1. 2,4-Dichlorophenol					
% Disappearance	1	0	0	15	0
	2	80	75	40	55
	3	80	85	50	60
	4	90	90	50	60
% Chloride Ion	1	23	23	6	3
	2	47	35	12	3
	3	47	47	12	3
	4	47	58	12	3
2. 2,4,6-Trichlorophenol					
% Disappearance	1	100	100	20	0
	2	100	100	60	0
% Chloride Ion	1	19	28	5	0
	2	19	28	5	0
3. Dichlorohydroquinone					
% Disappearance	1	90	100	100	0
	4	100			20
	5				45
% Chloride Ion	1	75	25	13	7
	4	100	50	31	19
	5	100	50	31	25

TABLE III

DATA FOR EFFECT OF REACTIVATION

Neutral pH (7) and Room Temperature (26°C)

Compound	Conc. mg/l	Per Cent Change in Days Aerated 1	2	3	4
1. ortho-Chlorophenol	100				
Reactivated					
% Disappearance		100	-	-	
% Chloride		0			
Not Reactivated					
% Disappearance		100	-	-	
% Chloride		0			
2. meta-Chlorophenol	100				
Reactivated					
% Disappearance		60	100	100	
% Chloride		36	36	89	
% Disappearance		95	100	100	
% Chloride		54	89	100	
Not Reactivated					
% Disappearance		60	100	100	
% Chloride		36	72	100	
3. para-Chlorophenol	100				
Reactivated					
% Disappearance		60	80	100	
% Chloride		18	36	100	
Not Reactivated					
% Disappearance		60	90	100	
% Chloride		0	36	100	
4. 2,4-Dichlorophenol	100				
Reactivated					
% Disappearance		90	90	90	
% Chloride		69	69	69	
% Disappearance		85	90	90	
% Chloride		46	80	80	

(continued)

TABLE III (Cont'd)

DATA FOR EFFECT OF REACTIVATION

Neutral pH (7) and Room Temperature (26°C)

Compounds	Conc. mg/l	Per Cent Change in Days Aerated 1	2	3	4
4. 2,4-Dichlorophenol (cont'd)	100				
Not Reactivated					
% Disappearance		60	70		
% Chloride		23	46	46	
5. 2,5-Dichlorophenol	100				
Reactivated					
% Disappearance		10	40		
% Chloride		0	0		
Not Reactivated					
% Disappearance		10	40		
% Chloride		0	0		
6. 2,4,6-Trichlorophenol	100				
Reactivated					
% Disappearance		0	20	60	
% Chloride		0	19	38	
% Disappearance		0	20	100	
% Chloride		0	19	47	
Not Reactivated					
% Disappearance		5	15	20	
% Chloride		5	10	10	

TABLE IV

ADDITIONAL DATA FOR EFFECT OF REACTIVATION

Neutral pH (7) and Room Temperature (26°C)

Compound	Conc. mg/l	Per Cent Change in Days Aerated					
		1	2	3	4	5	6
1. ortho-Chlorophenol	100						
Reactivated							
% Disappearance of Phenol*		100	100	100			
% Chloride Ion		15	15	15			
Not Reactivated							
% Disappearance		90	100	100			
% Chloride Ion		10	15	15			
2. meta-Chlorophenol	100						
Reactivated							
% Disappearance		35	80	100			
% Chloride Ion		35	80	100			
Not Reactivated							
% Disappearance		35	80	100			
% Chloride Ion		35	80	100			
3. para-Chlorophenol	100						
Reactivated							
% Disappearance		10	25	35			
% Chloride Ion		0	0	0			
Not Reactivated							
% Disappearance		10	15	40			
% Chloride Ion		0	15	15			
4. 2,4-Dichlorophenol	100						
Reactivated							
% Disappearance		0	20	40			
% Chloride Ion		0	0	34			
Not Reactivated							
% Disappearance		25	50	75			
% Chloride Ion		0	10	68			

(continued)

TABLE IV (Cont'd)

ADDITIONAL DATA FOR EFFECT OF REACTIVATION

Neutral pH (7) and Room Temperature (26°C)

Compound	Conc. mg/l	Per Cent Change in Days Aerated					
		1	2	3	4	5	6
4. 2,4-Dichlorophenol (cont'd)							
Not Reactivated (Separate test from above)							
% Disappearance						75	100
% Chloride Ion						50	100
5. 2,4,6-Trichlorophenol	100						
Not Reactivated							
% Disappearance				100	100		
% Chloride Ion				75	75		

*As observed by direct graphs in the ultraviolet.

the corresponding 100 per cent production of chloride ion. This change (which altered the dimensions of the aeration solution such that a better sludge distribution was obtained) yielded complete 100 per cent degradation of all the monochlorophenols and 2,4-dichlorophenol with the corresponding 100 per cent production of chloride ion and increased the partial degradation of the remaining compounds except sodium pentachlorophenate. Many of the compounds on various occasions have exhibited complete phenol disappearance (as indicated by spectrophotometric data), without the corresponding chloride ion production. Partial loss of the compound may be attributed to spattering or volatilizing effects of aeration.

An interesting aspect that evolved from this work was the observation that the ultraviolet spectrographs showed an increase in the concentration and/or change in the identity of the compound under conditions of no aeration. Two additional cylinders were used during several of the later tests. One of these cylinders contained the compound being studied (along with aerobic sludge and mineral nutrients) but the mixture was not aerated or stirred. The other cylinder was identical with the exception that it was magnetically stirred so that the sample received approximately the same agitation as those that were aerated.

Almost every sludge-compound mixture that was not aerated but was magnetically stirred showed an increase and/or change in the compound studied. The cylinders which were neither aerated nor stirred showed either compound stability or only a small increase and/or change.

This nonaerated supplementary work included observations on the ortho-

chlorophenol, meta-chlorophenol, para-chlorophenol and 2,4-dichlorophenol.

The concentration in the ortho-chlorophenol sample that was neither aerated nor stirred increased by 25 per cent the first day, decreased the second, and increased again on the third day (Figure 1). The samples of the ortho-chlorophenol that was magnetically stirred exhibited similar behavior (Figure 2). A comparison of the standard curve with the samples after 9 days showed a marked change in the configuration of the compound (Figure 3).

The behavior of meta-chlorophenol under the above conditions was similar to some extent. The sample that was magnetically stirred exhibited a fivefold increase on the second day with a small decrease on the third day (Figure 4). However, the meta-chlorophenol sample that was neither aerated nor stirred showed only very slight increases the first and second days with a very slight decrease the third day (Figure 5).

The parachlorophenol exhibited entirely different behavior from either of the two preceding compounds studied. The sample that was neither stirred nor aerated showed great stability (Figure 6). The sample that was magnetically stirred showed a slight decrease the first day with a slight increase the second and third days (Figure 7).

The two 2,4-dichlorophenol samples were almost identical in their behavior under conditions of no aeration with or without stirring. Each sample showed an increase in the concentration of a chlorinated phenol with some change in the identity of the compound (Figures 8 and 9).

The data showing an increase in the concentration of the chlorinated

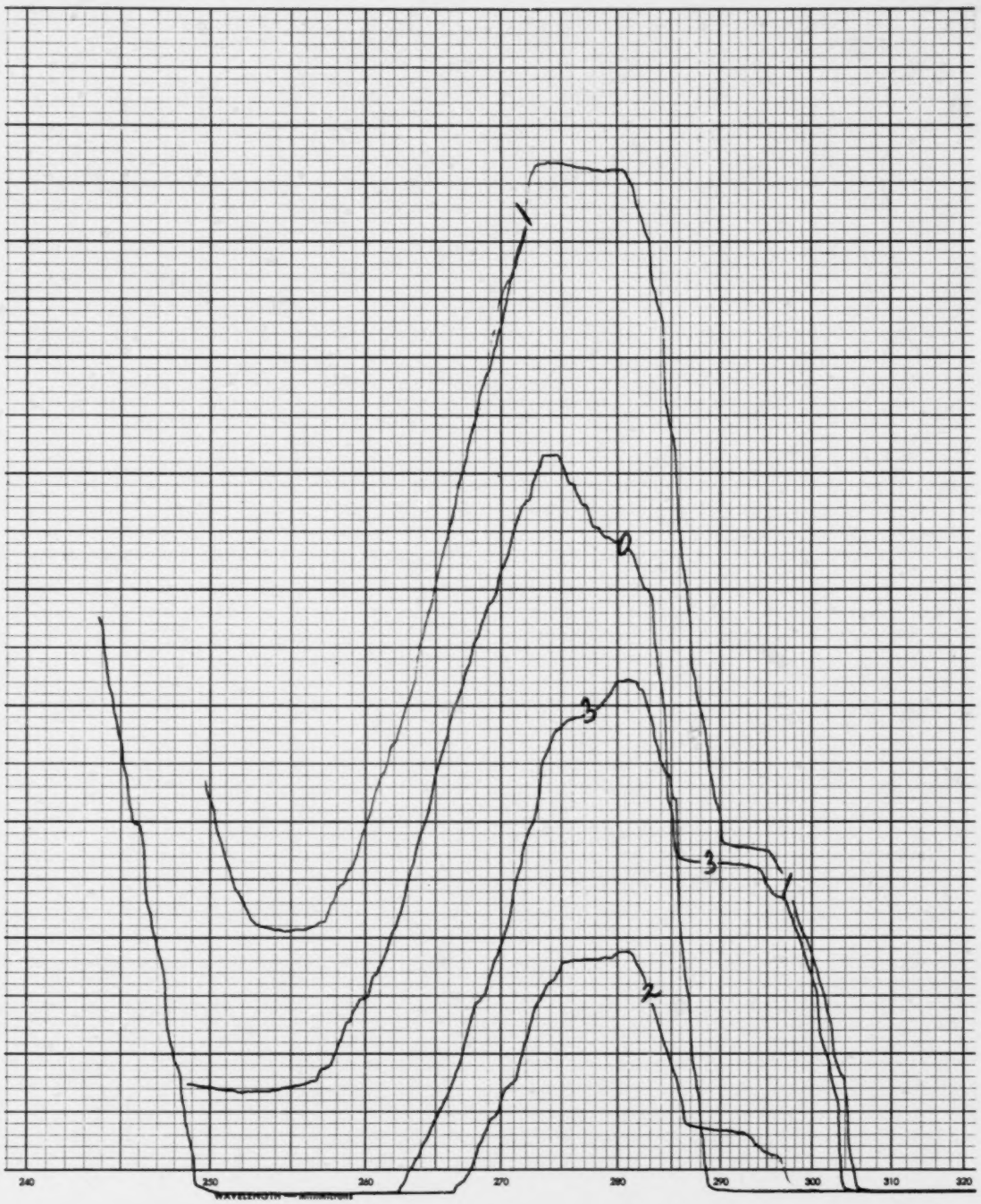

Figure 1. The Effect of Sludge Upon 100 mg/liter ortho-Chlorophenol which is neither Aerated nor Stirred.

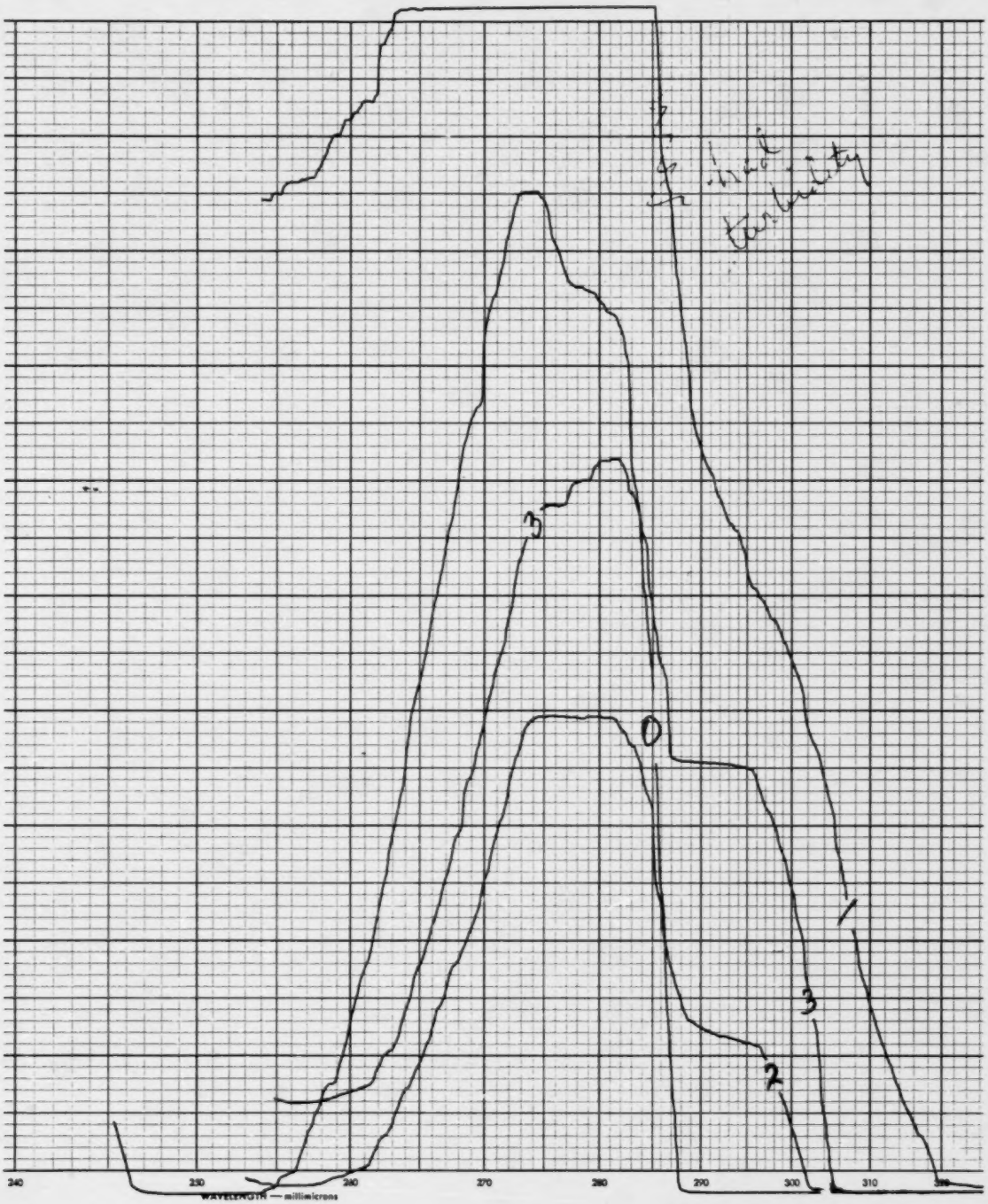

Figure 2. The Effect of Sludge Upon 100 mg-liter ortho-Chlorophenol, not Aerated but Magnetically Stirred.

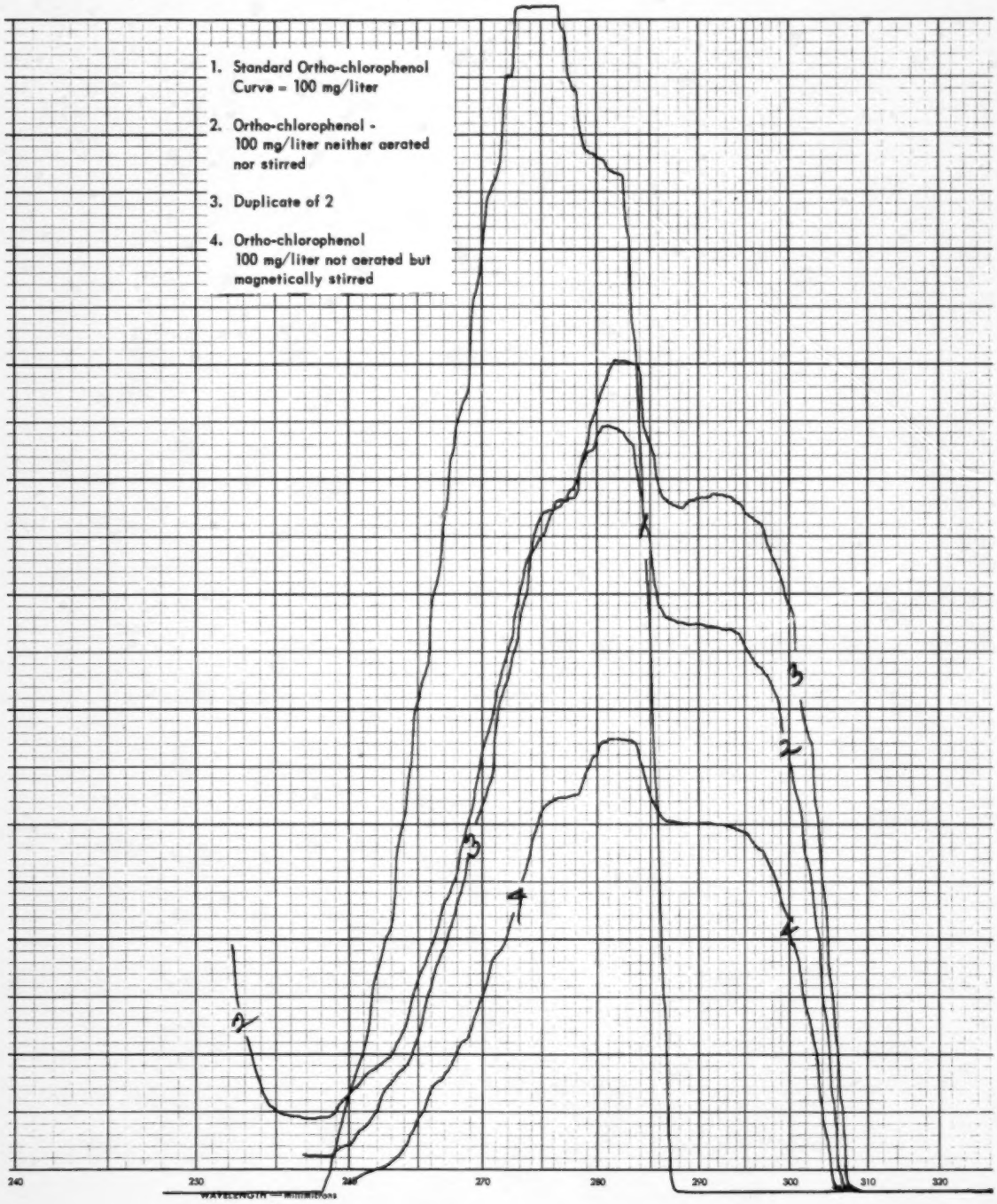

Figure 3. The Effect of Sludge Upon 100 mg/liter ortho-Chlorophenol after Nine Days.

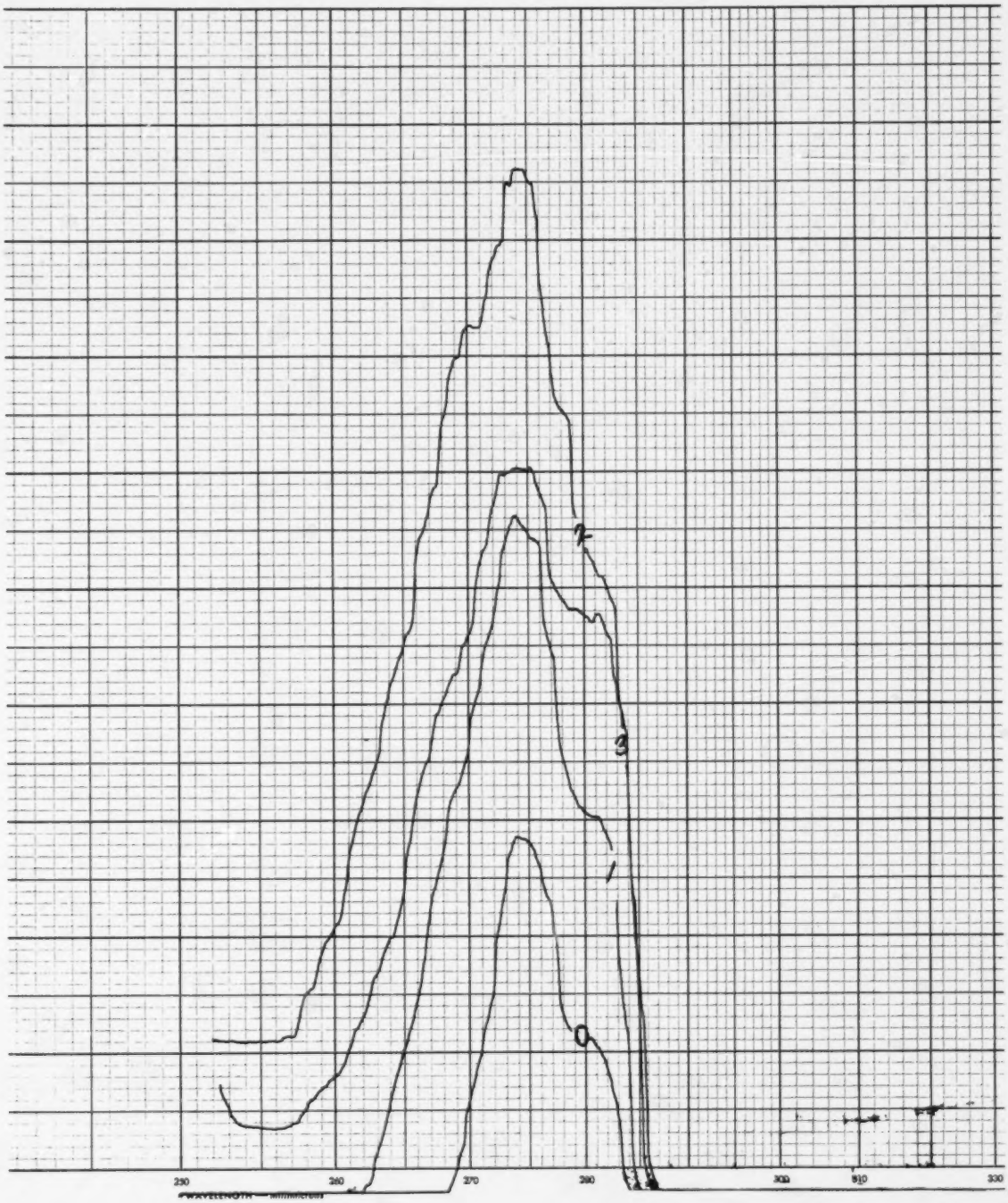

Figure 4. The Effect of Sludge Upon 100 mg/liter meta-Chlorophenol, not Aerated but Magnetically Stirred (less sensitive scale).

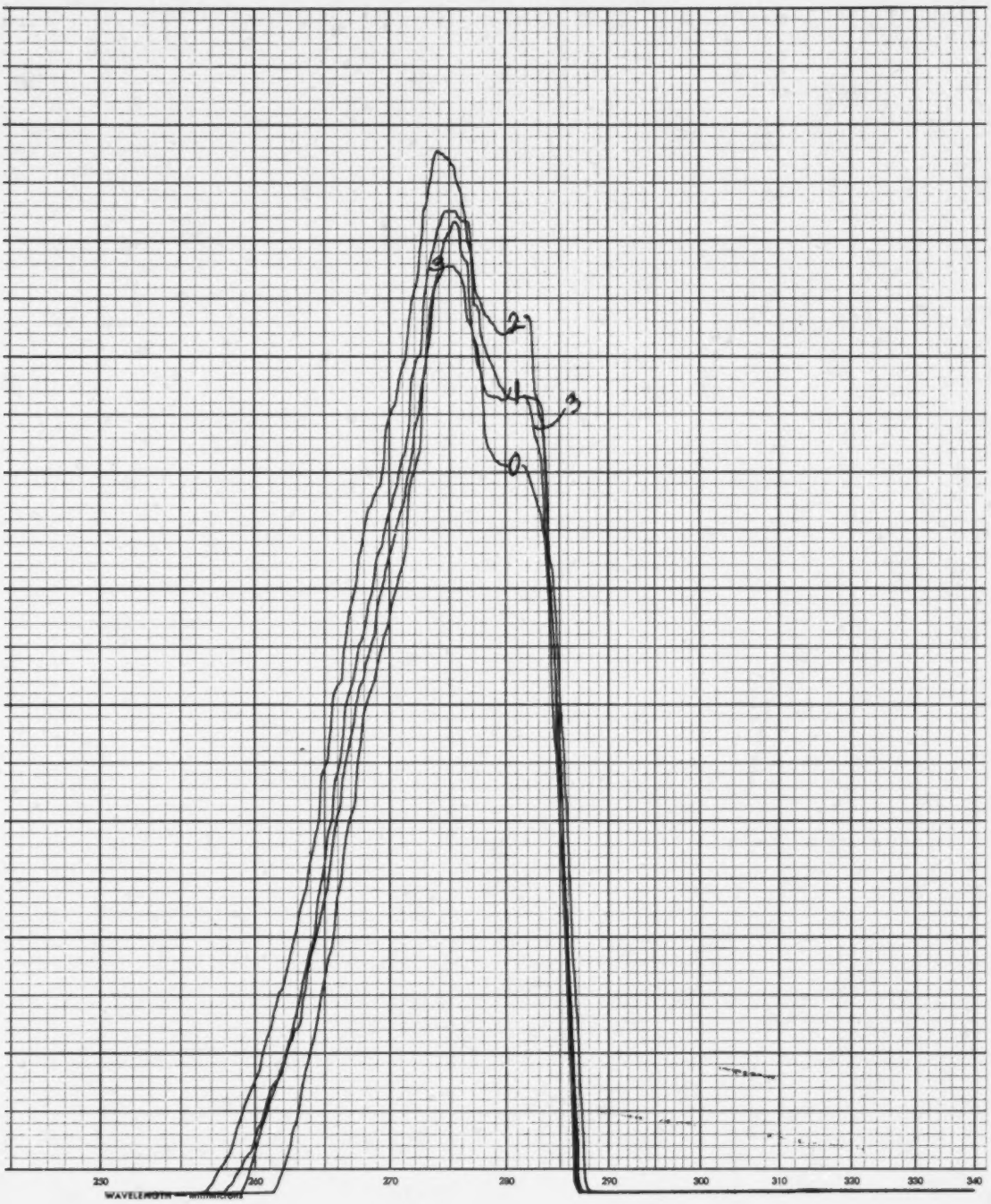

Figure 5. The Effect of Sludge Upon 100 mg/liter meta-Chlorophenol, neither Aerated nor Stirred.

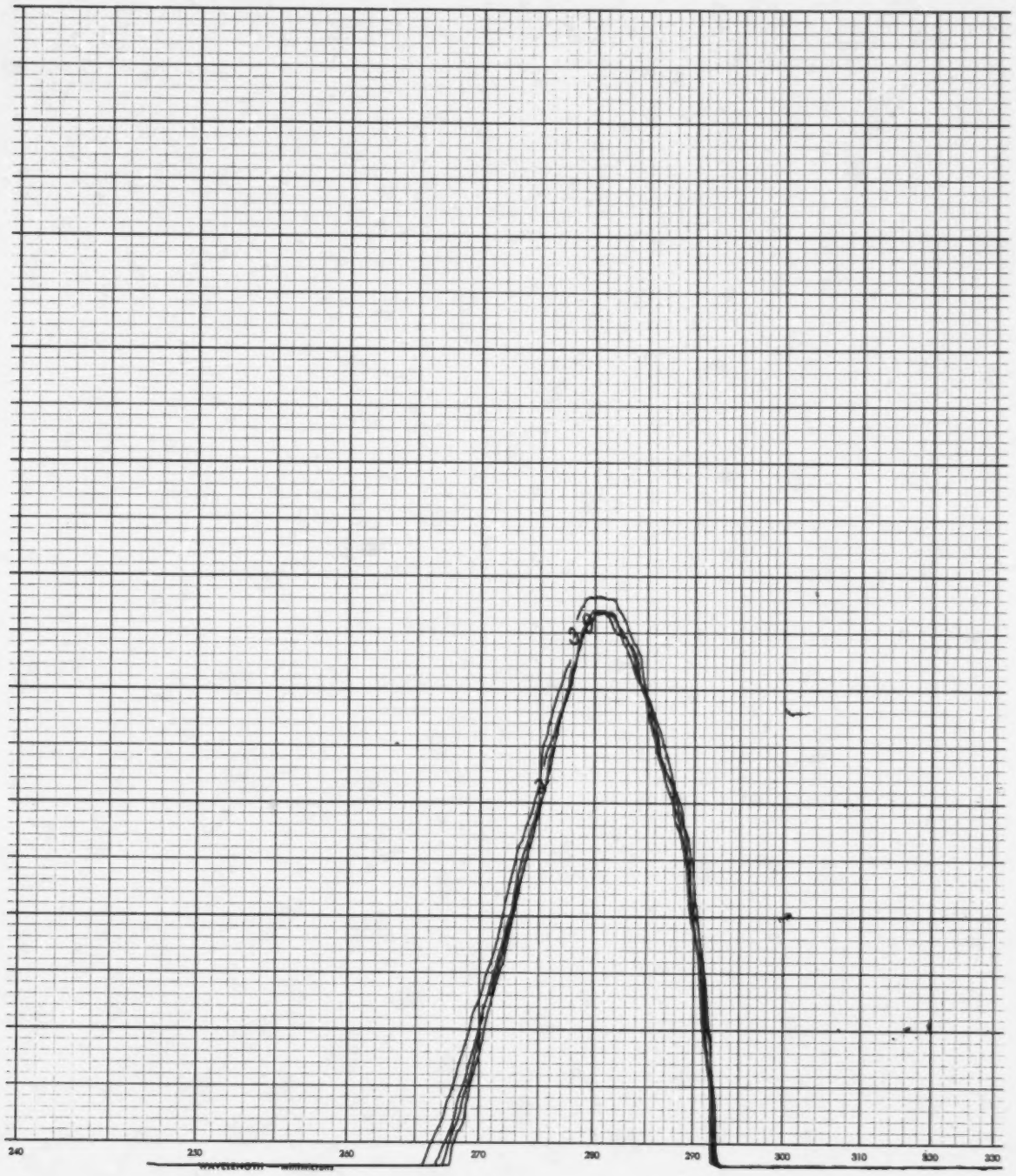

Figure 6. The Effect of Sludge Upon 100 mg/liter para-Chlorophenol, neither Aerated nor Stirred.

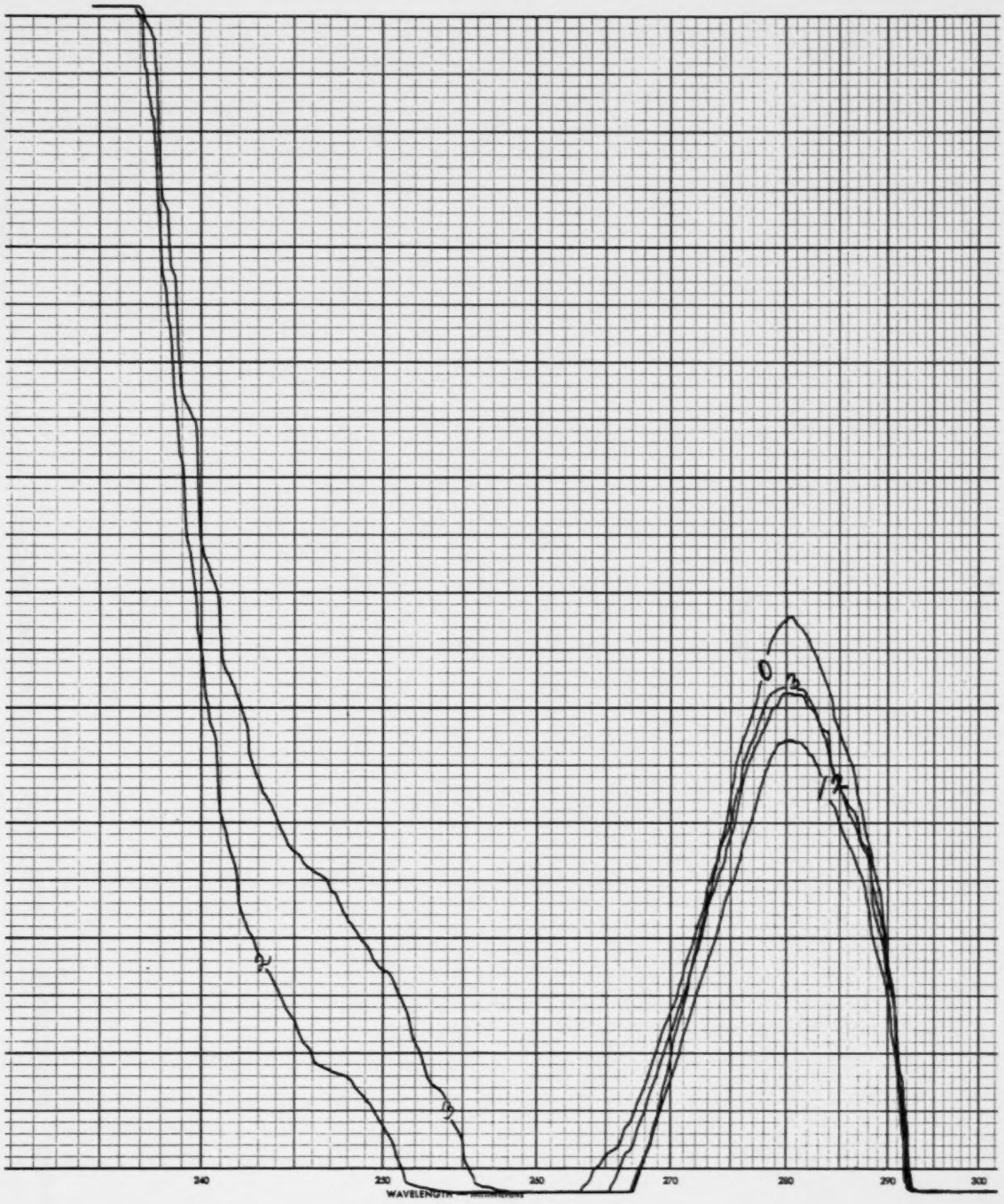

Figure 7. The Effect of Sludge Upon 100 mg/liter para-Chlorophenol, not Aerated but Magnetically Stirred.

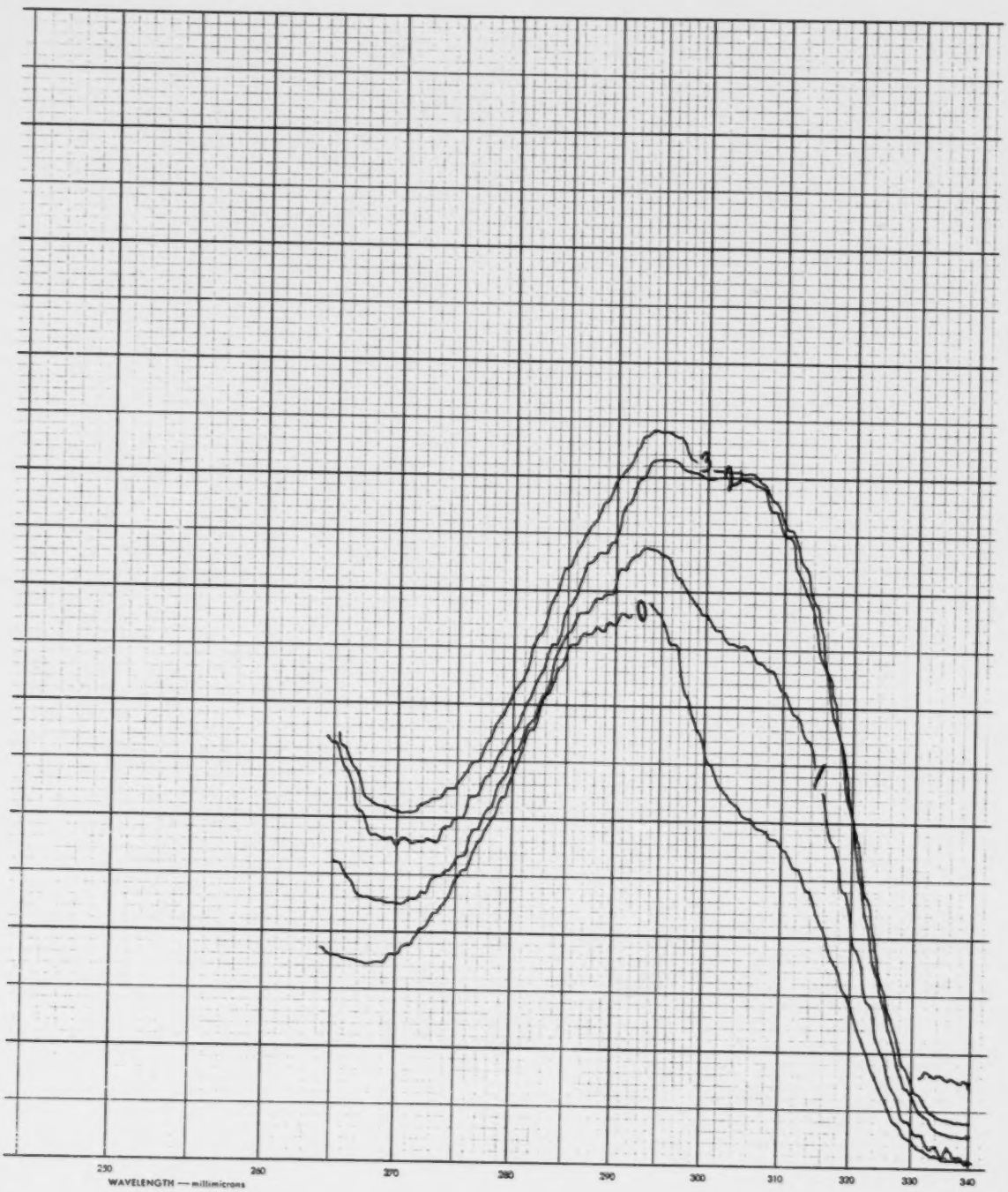

Figure 8. The Effect of Sludge Upon 100 mg/liter 2,4-dichlorophenol, neither Aerated nor Stirred.

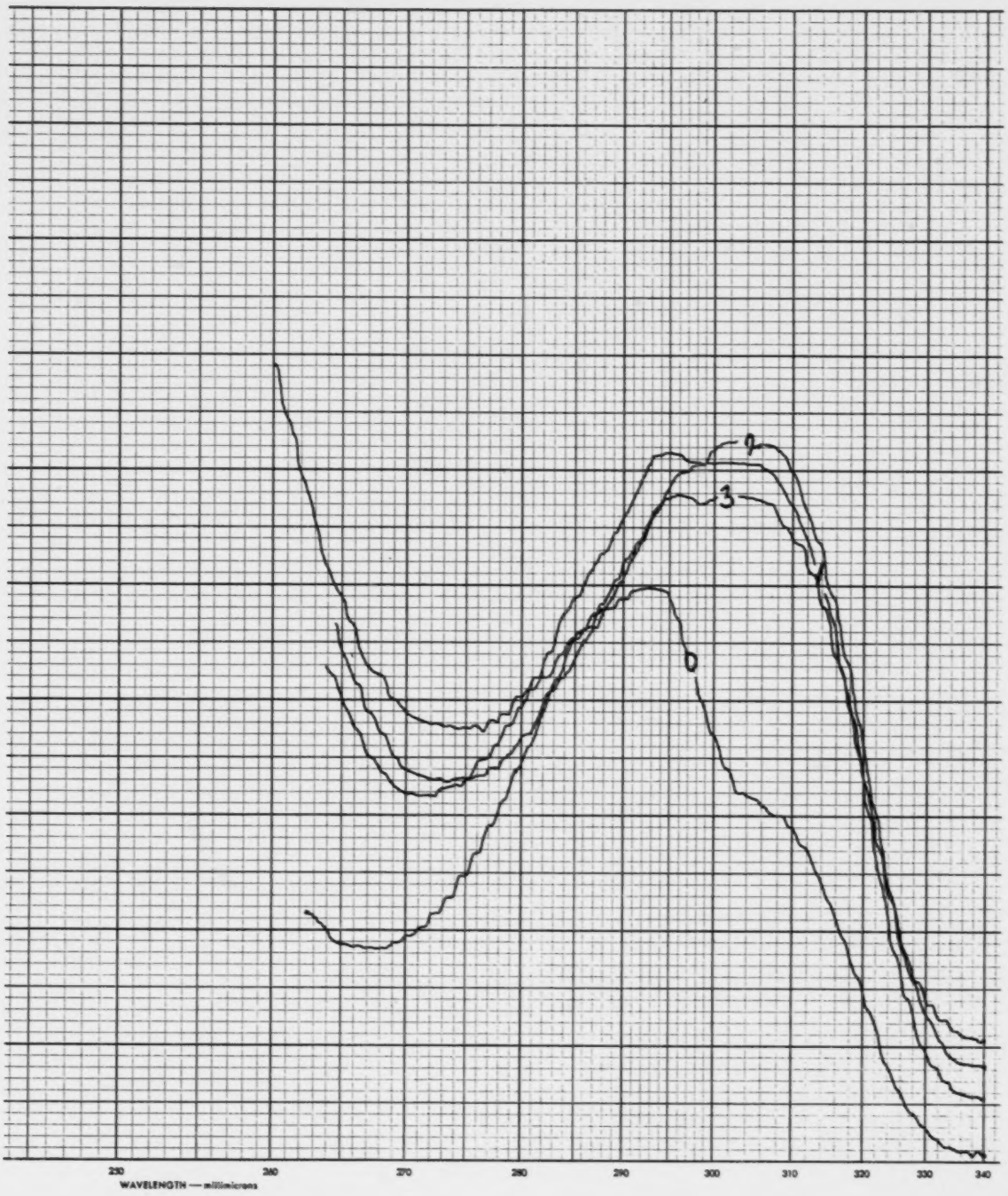

Figure 9. The Effect of Sludge Upon 100 mg/liter 2,4-dichlorophenol, not Aerated but Magnetically Stirred.

phenols when the sludge was stirred but not aerated indicate that these conditions favor either desorption of previously adsorbed compounds or a synthesis of some substituted phenols. The more likely desorption indicates the importance of oxygen in the degradation of the carbon-chlorine bond as well as the importance of measuring the chloride ion as the only sure way of indicating degradation.

The toxicity (reported as median tolerance level - TL_m as determined according to Standard Methods (9) of these compounds to fish has been found to have a general relationship to the ease of degradation of the various chlorinated phenols by bacteria, according to the results reported in Tables V and VI. Thus, pentachlorophenol is degraded only at low concentrations and is most toxic to fish with TL_m of 0.25 mg/l. The monochlorophenols with a TL_m of 10 mg/l without aeration are least toxic to fish and can be degraded readily at 100 mg/l.

The fact that the toxicity of the chlorophenols varies with the dissolved oxygen level, as shown in Figure 10, was completely unexpected. Since then it has been found that Edmunds and Gunn (1) have reported that the toxicity to fish of phenol itself is related to the dissolved oxygen level during the test. This would indicate that the mechanism of toxicity of the phenol and the chlorophenols may be similar. It is believed that phenol depresses the nervous system. Death by phenol results from asphyxia. The work of Edmunds and Gunn support our observations.

SUMMARY

Nine chlorinated phenols have been studied for their rate of biological

over
to p 28

TABLE V

MAXIMUM DEGRADATION TO DATE

Neutral pH (7) and Room Temperature (26°C)

Compound	Conc. mg/l	Per Cent Change after Days Aerated 1	2	3	4	5
1. ortho-Chlorophenol	100					
% Disappearance of ring*		45	80	100	100	
% Chloride Ion		18	36	36	100	
2. meta-Chlorophenol	100					
% Disappearance		95	100	100		
% Chloride Ion		54	89	100		
3. para-Chlorophenol	100					
% Disappearance		60	80	100		
% Chloride Ion		8	36	100		
4. 2,4-Dichlorophenol	100					
% Disappearance						100
% Chloride Ion						100
5. 2,5-Dichlorophenol	100					
% Disappearance		0	30	30	52	
% Chloride Ion		0	0	0	16	
6. 2,4,6-Trichlorophenol	100					
% Disappearance		0		100		
% Chloride Ion		0		75		
7. Na pentachlorophenate	100					
% Disappearance		0	0	0	0	
% Chloride Ion		0	0	0	0	
8. Dichloroquinone	100					
% Disappearance		100	-			
% Chloride Ion		25	25	50		
9. 2,5-Dichloro-para-benzoquinone	100					
% Disappearance		30	-			
% Chloride Ion		0				

*As observed by direct graphs in the ultraviolet.

TABLE VI

A SUMMARY OF RELATIVE TOXICITIES OF VARIOUS CHLOROPHENOLS

Compound	TL_m (24 Hr. at 20° C) mg/l
2,4-Dichlorophenol	6.0
2,5-Dichlorophenol	2.64
para-Chlorophenol	2.73
ortho-Chlorophenol	7.76
meta-Chlorophenol	4.30
2,4,6-Trichlorophenol	0.97
Pentachlorophenol	0.23

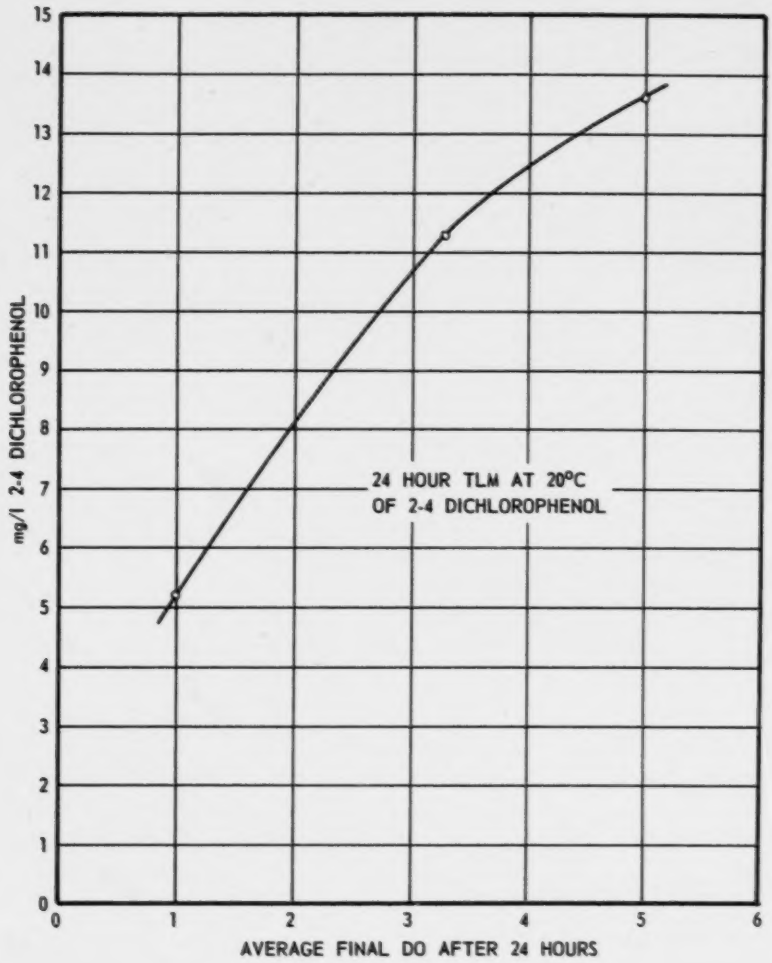

Figure 10. Correlation of Dichlorophenol Toxicity with Dissolved Oxygen at Termination of 24-Hour Test Period.

degradation. The disappearance of the phenol has been observed by recording changes in ultraviolet absorption. The cleavage of the carbon-chlorine bond has been determined by observing the increase in the chloride ion. Ortho-, meta-, and para-chlorophenol, and 2,4-dichlorophenol could be degraded completely in 3 to 4 days; 2,5-dichlorophenol, 2,4,6-trichlorophenol, dichloroquinone, and 2,5-dichloro-parabenzoquinone were only partially degraded during this time; sodium pentachlorophenate was not degraded.

There is some evidence that the decrease in concentration of the more biologically refractive compounds occurs by adsorption into the slime and that these compounds are not immediately degraded. When some sludge is mixed with agitation but without oxygen in the presence of more of the refractive compound, the concentration of the refractive compound in solution may increase sharply.

The toxicity of these compounds toward fish shows a rough correlation with the ease of bacterial degradation. The toxicity of these compounds to fish is greatly reduced by an increase in the dissolved oxygen level. This fact would indicate that death may result from the same mechanism reported for phenol--asphyxia from damage to the nervous system.

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